REMARKS

Status of the Claims

Claims 1-4, 6-9, and 13-24 are pending in the present application. Claims 5 and 10-12 are canceled. Claims 14, 17-19, and 22-24 are withdrawn as directed to a non-elected invention.

Claim 1 is amended for clarity and to cancel the identified subject matter. Claim 1 is further amended to specify that the cells are cultured in the presence of "IL-2." In addition, claim 1 is amended to specify that the cells are cultured in the "absence of an antigen-presenting cell comprising antigenic peptide on its surface." Support for amended claim 1 is found throughout the application as originally filed including the examples and on pages 37-38, bridging paragraph. Claims 3, 15, and 18 are also amended for clarity and to cancel the identified subject matter.

The claims are amended without prejudice or disclaimer. Applicants reserve the right to claim any canceled subject matter in one or more divisional or continuation applications.

No new matter is added by way of this amendment. Reconsideration of this application is respectfully requested.

Issues Under 35 U.S.C. § 112, First Paragraph

Precursor Cells

Claims 1-4, 6-9, 13, 15, 16, 20, and 21 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement, *see* Office Action, pages 2-7, items 3-4. Specifically, the Examiner alleges that only peripheral blood mononuclear cells are suitable precursor cells for differentiating cytotoxic lymphocytes, *see* Office Action, pages 2-5, item 4. According to the Examiner, umbilical cord blood mononuclear cells may not predictably be used to form cytotoxic lymphocytes. This rejection is respectfully traversed.

In response to Applicants' previous arguments, the Examiner states that D.L. Nelson *et al.*, *Pediatric Research*, 1986, 20: 136-139, ("Nelson"), teach that cord blood T cells fail to produce soluble IL-2R in response to anti-CD3 stimulation. The Examiner believes that this statement indicates that cord blood T cells might not be fully functional. In addition, the Examiner states Applicants have not provided any evidence demonstrating that producing cytotoxic lymphocytes from umbilical cord blood mononuclear cells was routinely performed in the art at the time of the invention.

Applicants do not agree with the Examiner. Nevertheless, in an effort to expedite prosecution, Applicants have amended the claims to cancel the subject matter, which the Examiner alleges is not supported by the present application. Accordingly, this aspect of the rejection is overcome.

IL-2

The Examiner further alleges that the claims fail to comply with 35 U.S.C. § 112, first paragraph, because IL-2 is an essential element of the claimed invention, *see* Office Action, pages 5-7, item 4. This rejection is respectfully traversed.

According to the Examiner, all of the working examples in the present application include IL-2. In addition, the Examiner states that the instant application teaches that IL-2 is an essential ingredient for obtaining cytotoxic lymphyctes.

In an effort to expedite prosecution, independent claim 1 is amended to specify IL-2. Accordingly, Applicants believe the rejection is overcome and respectfully request withdrawal.

Issues Under 35 U.S.C. § 112, Second Paragraph

Claims 1-4, 6-9, 13, 15, 16, 20, and 21 are rejected under 35 U.S.C. § 112, second paragraph, for an alleged lack of clarity, *see* Office Action, pages 7-8, item 6. This rejection is respectfully traversed.

Applicants have amended the claims to correct each of the deficiencies specifically pointed out by the Examiner. Applicants respectfully submit that the claims, as amended, particularly point out and distinctly claim the subject matter which Applicants regard as the invention.

Accordingly, reconsideration and withdrawal of this rejection are respectfully requested.

Issues under 35 U.S.C. § 103(a)

Darfler, Ochoa, Cardarelli, Taguchi, and, optionally, Chen

Claims 1-4, 6-9, 13, 15, and 16

Claims 1-4, 6-9, 13, 15, and 16 are rejected under 35 U.S.C. § 103(a) as allegedly obvious over PCT Publication No. WO 88/02774 to Darfler, ("Darfler"), in view of Ochoa *et al.*, *Cancer Res.*, 1989, 49:963-968, ("Ochoa"), Cardarelli *et al.*, *Cell Immunol.*, 1991, 135:105-117,

("Cardarelli"), and U.S. Patent No. 5,198,423 to Taguchi *et al.*, ("Taguchi"), *see* Office Action, pages 8-11, items 7-8.

In response to Applicants' previous arguments regarding the necessity of using a compound in Darfler for activating kinase C in order to obtain a serum-free medium, which has the equivalent function of a serum-containing medium, the Examiner states that the serum free medium, AIM-V, described in the working examples of the present application, includes diacylglycerol, which activates kinase C.

In regard to Applicants' comments regarding Cardarelli, the Examiner states that Cardarelli does not need to explicitly teach that anti-CD3, fibronectin and IL-2 produce cytotoxic lymphocytes from peripheral mononuclear cells to make the production of cytotoxic lymphocytes in the presence of fibronectin obvious. According to the Examiner, Cardarelli teaches that peripheral mononuclear cells, expanded in the presence of anti-CD3 and fibronectin induce expression of CD25. The Examiner believes that an ordinary artisan would have, accordingly, reasonably expected that anti-CD3, fibronectin, and IL-2 could be used in a method of making cytotoxic T cells from peripheral mononuclear cells since, not only would the cells be expanded to large numbers, but the cells would also be fully sensitive to the LAK-inducing effects of IL-2 *via* binding of IL-2 to CD25.

The claimed invention

As amended, independent claim 1 is directed to a method for preparing a cytotoxic lymphocyte which method comprises: a) culturing peripheral blood mononuclear cells wherein said peripheral blood mononuclear cells are capable of differentiating into cytotoxic lymphocytes with a medium containing serum and plasma at a total concentration of 0% by volume or more and less than 5% by volume, in the presence of a recombinant fibronectin fragment, which is a polypeptide comprising at least any one of the amino acid sequences shown in SEQ ID NOs: 1 to 20 and 25 of the sequence listing and IL-2, wherein said fibronectin fragment comprises a cell adhesion activity and/or a heparin binding activity, wherein a cytotoxic activity is enhanced as compared to a cytotoxic activity of a cytotoxic lymphocyte prepared in the absence of the recombinant fibronectin fragment, wherein the cells are cultured in the absence of an antigen-presenting cell comprising antigenic peptide on its surface, and wherein the method, optionally, further comprises, maintaining or expanding the cytotoxic lymphocytes by culturing the

cytotoxic lymphocytes obtained in step (a) with a medium containing serum and plasma at a total concentration of 0% by volume or more and less_than 5% by volume, in the presence of a recombinant fibronectin fragment, which is a polypeptide comprising at least any one of the amino acid sequences shown in SEQ ID NOs: 1 to 20 and 25 of the sequence listing and IL-2.

Unexpected Results

Applicants submit that the claimed methods result in effects that could not have been expected by an ordinary artisan at the time of the invention. In particular, as described on page 19, line 22 to page 20, line 20 in the originally filed application, the use of recombinant fibronectin in the claimed method results in, for example an enhanced level of cytotoxicity as well as increased cell proliferation. That is, the inventors have surprisingly discovered that recombinant fibronectin is more effective than native fibronectin in obtaining desirable features of the described cultured cells.

Applicants further submit that an ordinary artisan could not have reasonably expected from the cited references that substituting recombinant fibronectin for native fibronectin would result in enhanced levels of cytotoxicity and increased cell expansion. Cardarelli, for example, uses native fibronectin. Accordingly, Applicants submit that an ordinary artisan could not have expected the effects of the claimed methods from Cardarelli or any of the cited references, either alone or in combination.

Moreover, the Examiner appears to indicate that the induction of CD25 expression, as described in Cardarelli, is correlated with an increase in cytotoxicity levels. However, CD25 induction merely indicates that an IL-2 receptor is expressed. Applicants submit that the induction of CD25 would not have suggested to an ordinary artisan that cytotoxicity is enhanced. Furthermore, Applicants submit that the Examiner has failed to provide sufficient grounds for this assertion.

In addition, Applicants submit that an ordinary artisan could not have expected from the cited references that AIM-V medium, comprising a protein kinase C activating compound, in combination with a recombinant fibronectin fragment, would have resulted in increased levels of cell expansion. Applicants note that the Examiner correctly states that "AIM-V medium used in applicant's working examples contain the protein kinase C activating compounds", *see* Office Action, page 10. Nevertheless, at the time of the invention an ordinary artisan could not have

expected that using AIM-V serum-free medium, which includes a protein kinase C activating compound, in combination with fibronectin could have resulted in remarkably increased expansion of LAK cells, *see for example* Table 43 and Example 43 of the originally filed application.

In view of the foregoing, Applicants submit that the claims are not rendered obvious by the cited references. Accordingly, withdrawal of the rejection is respectfully requested.

Claims 20 and 21

Claims 20 and 21 are also rejected under 35 U.S.C. § 103(a) as allegedly obvious over Darfler, Ochoa, Cardarelli, Taguchi, and Chen *et al.*, *J. Immunol.*, 1994, 153:3630-3638 ("Chen"), *see* Office Action, page 11, item 9. These rejections are respectfully traversed.

As noted above, the claims are not rendered obvious by the combination of Darfler, Ochoa, Cardarelli and Taguchi. Applicants submit that Chen fails to remedy the deficiencies of these references.

Chen describes the transduction of PKC genes into cytotoxic T lymphocytes. The present invention does not specify this feature. Further, Applicants submit that the present application and the claimed invention are very different from the disclosure described in Chen. Applicants also submit that an ordinary artisan would not have been motivated to combine Chen with Darfler, Ochoa, Cardarelli and Taguchi to achieve the instantly claimed invention.

In view of the foregoing, Applicants believe the rejection is overcome and respectfully request withdrawal.

Sagawa, Johnson, Darfler, and Freshney

Claims 1-4, 6-9, 13, 15, and 16 are also rejected under 35 U.S.C. § 103(a) as allegedly being obvious in view of PCT Publication No. WO 03/016511 to Sagawa *et al.*, ("Sagawa"), in view of Johnson *et al.*, *J. Immunol.*, 1992, 148:63-71, ("Johnson"), Darfler, Freshney ed., IRL Press, Animal Cell Culture, A Practical Approach, 1986, pages 26-41, ("Freshney"), as evidenced by the teachings of U.S. Publication No. 2005/0042208, which is the national stage application of Sagawa based upon PCT Publication No. WO 03/016511 and Mazumder *et al.*, *Cancer*, 1984, 53:896-905, ("Mazumder"), *see* Office Action, pages 11-15, item 10. Please note that Wolf *et al.*, *Vox Sang*, 2005,88:249-255 ("Wolf") is also cited as evidence of Darfler.

Claims 20-21 are also rejected under 35 U.S.C. § 103(a) as allegedly being obvious over Sagawa, in view of Johnson, Darfler, Freshney, as evidenced by Sagawa and Mazumder, and Chen, *see* Office Action, page 15, item 10. These rejections are respectfully traversed.

Basis for the rejection

In response to Applicants' previous arguments, the Examiner states that the claimed methods encompass antigen-presenting cells. The Examiner also states that Sagawa teaches methods for maintaining or expanding cytotoxic T cells, which do not require an antigen-presenting cell.

The claimed invention could not have been predictably achieved from the cited references

As amended, the claims specify that the cells are cultured in the absence of an antigen-presenting cell comprising antigenic peptide on its surface. As noted above, support for this amendment is found, for example, on pages 37-38, bridging paragraph, of the originally filed application. Applicants note that, if alternative elements are positively recited in the specification, they may be explicitly excluded from the claims, *see* MPEP 2173.05(i).

Applicants submit that Sagawa fails to describe preparing cytotoxic lymphocytes from peripheral blood mononuclear cells without the use of antigen-presenting cells and peptides. In addition, the disclosure to which the Examiner refers is limited to the maintenance or expansion of cytotoxic T cells. Accordingly, an ordinary artisan could not have reasonably predicted from Sagawa that cytotoxicity is enhanced by culturing peripheral blood mononuclear with fibronectin and serum free medium in the absence of an antigen-presenting cell comprising antigenic peptide on its surface.

Moreover, Sagawa teaches that cell expansion is achieved by inducing antigen-specific cytotoxic lymphocytes and then, subsequently, applying recombinant fibronectin (CH-296) to the cells. Accordingly, Sagawa's method, unlike the instantly claimed method, first requires induction of antigen-specific cytotoxic lymphocytes in the presence of an antigen-presenting cells, followed by cell expansion with recombinant fibronectin. Therefore, the "starting materials" used in Sagawa's method are distinguishable from those in the claimed methods. Accordingly, Applicants submit that the claimed invention could not have been predictably

achieved from the teachings in Sagawa. Neither Johnson, Darfler, Freshney nor Chen, in any combination with Sagawa, remedy these deficiencies.

Unexpected Results

Moreover, at the time of the invention an ordinary artisan recognized from related prior art that it is difficult to obtain adequate numbers of cytotoxic T-lymphocytes from peripheral blood mononuclear cells using serum-free medium. However, the present invention is characterized by increasing the efficiency of serum-free medium by using a recombinant fibronectin fragment.

As shown in Table 43 of working example 43 of the originally filed application, peripheral blood mononuclear cells, cultured in AIM-V serum-free medium, including protein kinase C activating compound, and IL-2, results in an expansion fold, in the presence of a recombinant fibronectin fragment, which is remarkably greater than the expansion fold observed in the absence of recombinant fibronectin fragment. That is, in the present invention, a recombinant fibronectin fragment in combination with the described serum free medium does not exhibit the same effect as medium containing serum or any comparable substance, but instead results in a remarkably increased effect on cell expansion.

As disclosed in Darfler, applying serum-free medium is useful for obtaining cells for use in adoptive immunotherapy. However, it is difficult to obtain the required number of cells for actual medical treatment using serum-free medium. In contrast to this teaching, the present inventors have been able to unexpectedly achieve sufficient numbers of cells using the claimed methods, *see* page 5, line 10 to page 6, line 4 of the originally filed application.

In view of the foregoing, the claims are not rendered obvious by the cited references. Withdrawal of the rejection is respectfully requested.

Parker and Bagnis

Claims 1-4, 6-9, 13, 15, 16, 20, and 21 are rejected under 35 U.S.C. § 103(a) as allegedly obvious over Parker *et al.*, *Hum Gene Ther.*, 2000, 11:2377-2387 ("Parker"), PCT publication No. WO 99/05301 to Bagnis *et al.* ("Bagnis"), as evidenced by U.S. Patent No. 6,287,864, which is the English language U.S. national stage entry of WO 99/05301, and as further evidenced by the Gibco/Invitrogen Publication, 2003, pages 1-7 ("Gibco") and Kaldjian *et al.*, *J. Immunol*

Methods, 1992, 147:189-195 ("Kaldjian"), *see* Office Action, pages 15-17, items 11-12. This rejection is respectfully traversed.

According to the Examiner, Parker describes preparing cytotoxic lymphocytes by culturing peripheral mononuclear cells in serum-free medium, IL-2, and anti-CD3 antibody. The Examiner admits that Parker does not teach culturing the cells with a recombinant fibronectin fragment. However, the Examiner believes that Bagnis remedies this deficiency. The Gibco publication and Kaldjian are cited for teaching that AIM V is serum free medium.

Parker employs serum-containing medium in a gene transfer process. Bagnis also applies recombinant fibronectin fragment in a gene transfer process, but not for cell culture. In this light, the claimed methods, Parker and Bagnis are distinguishable. Furthermore, in regard to cell culturing, after day 5 Parker teaches that T-cells are cultured without a recombinant fibronectin fragment. In contrast, the claimed methods use a recombinant fibronectin fragment when "culturing" peripheral blood mononuclear cells in order to induce cytotoxic T lymphocytes, which is one of the features of the present invention. The claimed methods do not use recombinant fibronectin fragment in a gene transfer process. In view of the foregoing, the combination of Parker, and Bagnis are remarkably different in terms of technique, from the claimed methods. Accordingly, Applicants submit that an ordinary artisan could not have achieved the instantly claimed methods from the combination of Parker and Bagnis. Further, Applicants submit that citing these references in the office action is improper, and no one of ordinary skill in the art would be motivated to combine these references.

In view of the foregoing, Applicants believe the rejection is overcome and respectfully request withdrawal.

Non-Statutory Obviousness Type Double Patenting

Claims 1-4, 6-9, 13, 15, 16, 20, and 21 are provisionally rejected on the ground of nonstatutory double patenting as allegedly obvious over claims 1-3, 5-7, 10, 12, 28, 29, 31-35, and 37-39 of co-pending U.S. Application No. 10/509,055 in view of Johnson, Darfler and Freshney, *see* Office Action, pages 17-18, items 13-14.

According to the Examiner, the cited claims fail to describe medium containing less than 5% serum. However, the Examiner asserts that Johnson, Darfler and Fresheny remedy this deficiency.

As noted above, Applicants submit that an ordinary artisan could not have reasonably expected that the cytotoxicity of differentiated peripheral mononuclear cells could have been enhanced by incubating the described precursor cells with a combination of recombinant fibronectin fragment and serum free medium in view of Johnson, Darfler and Freshney. Accordingly, Applicants believe the rejection is overcome and respectfully request withdrawal.

CONCLUSION

In view of the above amendment and remarks, Applicants believe the present application is in condition for allowance.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Linda T. Parker, PhD, Registration No. 46,046, at the telephone number of the undersigned below to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Director is hereby authorized in this, concurrent, and future replies to charge any fees required during the pendency of the above-identified application or credit any overpayment to Deposit Account No. 02-2448.

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Respectfully submitted,

By _____

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